

# LABELING GC-GLOBULIN WITH FLUORESCCEIN

## Materials

1. 2 mM PIPES, pH 7.0, 500 ml.
2. Gc-globulin (Calbiochem 345802), 1 mg.
3. CFSE (Carboxyfluorescein succinimidyl ester; Molecular Probes).
4. Lysine, 100 mM in buffer 1, 10 ml.
5. G25-150 desalting column, 1 cm diameter. Equilibrated with buffer 1.
6. Centricon-30.
7. PBS solution A, 200 ml.

## Procedure

1. Dissolve 1 mg Gc-globulin in 200 ul buffer 1, dialyze overnight at 4°C.
  2. Transfer Gc-globulin into a small vial with a flea bar.
  3. Dissolve ~1 mg CFSE in DMSO at a concentration of 50 mg/ml. Dilute 1:10 into buffer 1.
  4. Mix 50 ul CFSE with the Gc-globulin solution while stirring. Let react at room temperature for 150 min in the dark (without stirring).
  5. Stop reaction by addition lysine to 10 mM.
  6. Desalt in a G25 column, collect fluorescent fractions in the void volume.
  7. Concentrate with Centricon-30.
  8. Clarify in a 42.2Ti rotor at 25,000 rpm, 4°C for 30 min. Measure concentration with Lowry assay.
  9. Dialyze into PBS solution A, can be stored at 4°C for several weeks with 0.01% NaN<sub>3</sub>, or frozen in liquid N<sub>2</sub>.
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